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Microbial control of *Meloidogyne incognita* on *Capsicum chinense* under an organic production system

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ABSTRACT

Background/objective. Habanero pepper (*Capsicum chinense*) cultivation is limited by root-knot nematodes such as *Meloidogyne incognita*, and its control is achieved through the repeated application of synthetic nematicides. An alternative is the application of microbial agents. The objective of this study was to evaluate the control of *M. incognita* with native *Trichoderma* spp. in *C. chinense,* in an organic production system.

Materials and Methods. Seedlings of the 45-day-old Izamal cultivar were used and transplanted with a substrate based on soil, bocashi and volcanic rocks (6:3:1), three inoculations of native strains of *Trichoderma asperellum* (Ta13-17), *T. erinaceum* (Te10-15) and their combination were applied at the time of transplantation, and eight and 15 days later, as control treatments: a nematicide (Vydate®) and water. Four destructive samplings were carried out during the crop cycle, and the variables were the severity with the galling index, number of eggs and females per g of root.

Results. In relation to the control, the combination of *T. asperellum* (Ta13-17) and *T. erinaceum* (Te10-15) caused significantly lower severity of the nematode estimated with the AUDPC. The apparent infection rate using the Weibull model (1/b) and final galling index (4.13%), also showed the lowest average reproduction of the nematode (number of eggs and females per g of root). With the combination of *T. asperellum* (Ta13-17) and *T. erinaceum* (Te10-15) the crop yield was significantly improved by 47.26 and 34.25 % in relation to the control and Vydate®, respectively.

Conclusion. In an organic production system of habanero pepper for the control of *M. incognita*; the individual application of *T. asperellum* (Ta13-17) significantly decreased the gall index; however, when combined with *T. erinaceum* (Te10-15) it improved nematode control (86.17%).

Keywords: Habanero pepper, *Trichoderma asperellum*, *T. erinaceum*, Effectiveness, Phytonematode

INTRODUCTION

Vegetable crops in tropical and subtropical areas largely depend on proper management of root-knot nematodes (Sikora and Fernández, 2005; Ulloa *et al.*, 2016), especially *Meloidogyne* spp., which are difficult to control due to their wide host range, high reproduction rates, and parasitic lifestyle (Manzanilla *et al.*, 2004; Guerrero-Abad *et al.*, 2021).

In general, *M. incognita* induces the formation of galls (hypertrophy and hyperplasia) in roots, which block the flow of water and nutrients. As a result of vascular system malfunction, plant growth is reduced, causing chlorosis, wilting, flower and fruit drop, and yield losses (Hernández *et al.*, 2011). Management of these nematodes has relied on the use of organophosphate and carbamate nematicides (Marbán and Manzanilla, 2012). However, due to their toxicity and environmental persistence, there is an increasing focus on evaluating alternative control methods compatible with agroecosystem health (Xie *et al.*, 2015), such as *Trichoderma* spp., which act as antagonists of root pathogens (Szabó *et al.*, 2013).

The efficiency of *Trichoderma* spp. in regulating populations of root parasites such as nematodes depends mainly on the strain origin, its interaction with the host, its ability to adapt to the environment where it is introduced, and the type of nematode parasitism (Zhang *et al.*, 2014). Research on the use of antagonistic microorganisms is steadily increasing (Hallman *et al.*, 2009; Corazon-Guivin *et al.*, 2024).

Among the microorganisms that parasitize nematodes, fungi are especially promising because they have shown strong potential (Martínez-Canto *et al.*, 2023). *Trichoderma* spp. are active mycoparasites that have been widely studied for the biocontrol of fungi causing foliar and root diseases (Martínez-Canto *et al.*, 2021; Natsiopoulos *et al.*, 2024). Based on this, the objective of this study was to evaluate, under protected conditions, the individual and combined application of two *Trichoderma* species on *Capsicum chinense* for the control of *Meloidogyne incognita*.

MATERIALS AND METHODS

This study was conducted under protected conditions (greenhouse) and in the Phytopathology Laboratory of the Tecnológico Nacional de México/Campus Conkal, located on Avenida Tecnológico S/N, between 21°02’ and 21°08’ N latitude and 89°29’ and 89°35’ W longitude.

Experiment setup. Forty-five-day-old seedlings of habanero pepper cv. Izamal (red at full maturity) were transplanted into 5-kg bags filled with a substrate composed of soil, bocashi, and volcanic rock in a 6:3:1 ratio. The bags were arranged in a randomized complete block design inside a tunnel-type greenhouse with a plastic roof cover and walls lined with anti-aphid mesh. Five treatments were established: 1) *T. asperellum* (Ta13-17), 2) *T. erinaceum* (Te10-15), 3) a combination of *T. asperellum* (Ta13-17) and *T. erinaceum* (Te10-15), and two controls: 4) Vydate® (a.i. oxamyl 24% of chemical synthesis) applied at 2 mL L^{-1} of water at the time of transplanting, and 5) an untreated control, consisting of plants without nematode management.

Isolation of *Trichoderma* spp. strains and inoculation in pepper plants. The *Trichoderma* strains evaluated were provided by the Fungal Culture Collection of the

Phytopathology Laboratory at Campus Conkal, identified as *T. asperellum* (Ta13-17) (Celis-Perera *et al.*, 2021) and *T. erinaceum* (Te10-15) (Martínez *et al.*, 2021). For use, the strains were reactivated on potato dextrose agar (PDA) medium and incubated at 28 °C for 15 days. The fungal inoculum was then prepared (1×10^3 spores mL^{-1}). Before transplanting, this inoculum was applied separately and in combination to the soil, followed by two additional inoculations at eight and 15 days after transplanting.

Preparation of *Meloidogyne incognita* inoculum and plant inoculation. From established populations of *M. incognita* maintained under protected conditions in *Solanum lycopersicum* crops, galled roots were collected and dissected to obtain nematode egg masses. These were then surface-disinfested to recover nematode eggs. Disinfestation was carried out with 2% sodium hypochlorite for two minutes, followed immediately by successive rinses with tap water using sieves with mesh numbers 45, 100, 200, 325, and 400 (Ayoub, 1980). Nematode egg inoculation was applied to the substrate contained in 5-kg bags, with each bag inoculated with 500 larvae eggs (Martínez *et al*., 2023).

Variables for estimating *M. incognita* control. To assess treatment effectiveness, the variables of galling severity and reproduction were evaluated at 56, 90, 122, and 137 days after transplanting (dat). For severity, a six-class gall index scale was used (Taylor and Sasser, 1978). The midpoint of each class was used to perform the following analyses with epidemiological parameters: Area Under the Disease Progress Curve (AUDPC), apparent infection rate using the inverse of parameter b (1/b) with the Weibull model, and final severity using the Yfinal parameter (Pennypacker *et al.*, 1980; Campbell and Madden, 1990).

To evaluate the effect of treatments on *M. incognita* reproduction, eggs and females were counted per gram of root. Habanero pepper roots were fragmented, homogenized, and two grams were sampled. One gram was blended for 10 s with 20 mL of chlorine and 30 mL of tap water, then filtered through sieves with mesh sizes 45, 100, 200, 325, and 400. The collected eggs were washed with running water and counted using a nematode counting chamber under a compound microscope at 4× magnification. The other gram of root was stained with pre-prepared acid fuchsin by placing 1 g of root in a flask, adding 2 mL of acid fuchsin with 50 mL of tap water, and heating to boiling. After cooling, roots were rinsed with tap water to remove excess stain, and glycerin was added for preservation and later evaluation. Females were separated and counted from the stained roots under a stereomicroscope (Moo *et al*., 2018; Martínez *et al*., 2023).

Agronomic variables. In each of the four samplings (56, 122, and 137 dat), variables associated with crop productivity were measured. Plant height was recorded after removing plants from the bags and separating shoots from roots. Using a measuring tape, the distance from the plant apex to the stem base was measured. For leaf and stem dry weight, samples were placed in kraft paper bags and dried in an oven at 50 °C for 10 days until constant weight was reached. Root dry weight was obtained after washing the roots and evaluating disease severity, followed by drying, using the same procedure as for stems and leaves. Root volume was determined by water displacement using a 1000 mL graduated cylinder. Yield was estimated from eight harvests during the crop cycle, recording fruit weight, polar diameter, and equatorial diameter.

Results analysis. Epidemiological and nematode reproduction analyses, as well as agronomic variables, were processed using SAS software version 9.4 for Windows. For nematode reproduction variables, analyses of variance (ANOVA) were performed at the specified sampling dates. Mean comparisons were carried out using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Microbial control effect of *M. incognita* in habanero pepper. During the first sampling at 56 dat, the combined treatment of *T. asperellum* (Ta13-17) and *T. erinaceum* (Te10-15) showed the lowest gall index, a trend that continued through the final sampling at 137 dat, with an 86.17% reduction. This was followed by *T. asperellum* (Ta13-17) alone with a 65.26% reduction, while the untreated control without fungal inoculants showed the highest nematode damage, averaging 29.88% (Figure 1). Similar results were reported by Moo-Koh *et al.* (2018), where the interaction between two strains, *T. citrinoviride* (Th33-58) and *T. harzianum* (Th33-59), reduced root damage severity by 83%. Likewise, Affokpon *et al.* (2011) reported a lower gall index with *T. asperellum* (T-12) at nine weeks compared with the uninoculated control.

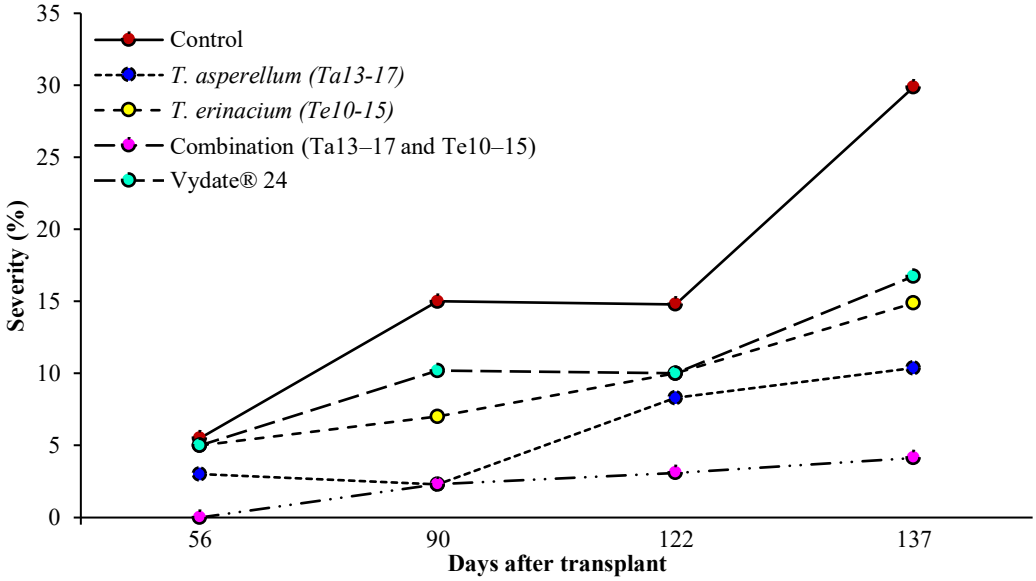

Figure 1. Curves of the progress of the gall index in the organic cultivation of *C. chinense*, during the period of 56, 90, 122 y 137 days after transplant.

Microbial control effect on epidemiological parameters. The AUDPC values showed that the combination of *T. asperellum* (Ta13-17) and *T. erinaceum* (Te10-15) resulted in the lowest disease progress, with 138.19 unit % day^{-1}, followed by *T. asperellum* alone with 376.94 unit % day^{-1}. This indicated that disease control was improved in these two treatments compared with the control. In an integrated management study of root-knot nematodes (*Nacobbus aberrans* and *M. incognita*) using biosolarization and *T. viride*, 457 unit % day^{-1} was recorded (Magallanes, 2021), also showing that the accumulation of organic matter in association with antagonistic and saprophytic organisms can reduce *M. incognita* populations (Pérez *et al.*, 2019).

Disease progression was fitted to the Weibull model, which allowed estimation of the apparent infection rate through the inverse of parameter b (1/b) (Pennypacker *et al.*, 1980). The treatments that resulted in the lowest apparent infection rates (disease progression speed) were the combination *T. asperellum* (Ta13-17) / *T. erinaceum* (Te10-15), Vydate®, and *T. erinaceum*, demonstrating that the application intervals were suitable for disease control. In contrast, the untreated control showed the highest disease intensity (Table 1). At the end of the experiment, the Yfinal parameter showed that the combination of Ta13-17 and Te10-15 provided the best disease control, with a final severity of 4.13%, which was significantly lower ($p \leq 0.01$) than the other treatments, all of which showed at least 30% final disease severity (Table 1). The antagonistic effect of *Trichoderma* spp. in nematode control is associated with their mechanisms of action, including the production of secondary metabolites such as viridin, gliotoxin, and gliovirin, which inhibit not only fungal growth but also egg hatching and the mobility of nematode juvenile stages (Zin and Badaluddin *et al.*, 2020) (Table 1).

Table 1. Effect of treatments on the control of *M. incognita* estimated with epidemiological parameters in the organic cultivation of *C. chinense*.

Treatment	AUDPC (unit % day^{-1})	Apparent infection rate *1/b* (unit % day^{-1})	r^2 (adjustment of the modelo Weibull)	Y_{final} (%)
T. asperellum (Ta13-17)	376.94 c [z]	0.0076 ab	0.97	10.38 ab
T. erinaceum (Te10-15)	778.25 b	0.0072 b	0.91	14.88 ab
Combination	138.19 c	0.0044 c	0.93	4.13 b
Control	1202.63 a	0.0091 a	0.90	29.88 a
Vydate®	815.75 b	0.0074 b	0.90	16.75 ab
DMS*	293.86	0.0016	-	11.56

[z]Means with the same letters are not statistically different (Tukey, ≤0.05). *Minimal Significant Difference ($P \leq 0.05$).

Number of eggs per gram of blended root. At 56 days after transplanting, the combination (Ta13-17 and Te10-15) showed no eggs, and maintained control of this variable with reductions of 81% at 122 days and 77% at 137 days after transplanting compared with the control (Table 2). Moo-Koh *et al.* (2018) evaluated the interaction of *T. simmonsii* (Th09-06) / *T. harzianum* (Th33-59) and achieved a 59.3% reduction in egg numbers. The treatment with *T. asperellum* alone caused a 37.65% reduction in egg numbers at 137 days after transplanting. Similarly, when *T. asperellum* strain Ta.90 was applied to tomato plants, egg numbers decreased by 50% (Hernández *et al.*, 2015). The reduction in egg numbers is a consequence of parasitism detected in egg masses and eggs, confirming the effect observed under *in vitro* conditions, where *T. asperellum* FbMi6 inhibited egg hatching and caused mortality of *M. incognita* juveniles (Saharan *et al.*, 2023).

Table 2. Effect of treatments on the number of *M. incognita* eggs in organic cultivation of *C. chinense*.

Treatment	Number of eggs per gram of liquefied root			
	56 dat [y]	90 dat	122 dat	137 dat
T. asperellum (Ta13-17)	70.25 bc [z]	86.50 b	1214.25 a	1361.75 ab
T. erinaceum (Te10-15)	105.75 bc	491.75 ab	1372.25 a	1487.25 ab
Combination	0.00 c	55.50 b	335.25 a	495.25 b
Control	394.25 a	944.25 a	1787.00 a	2184.25 a
Vydate®	302.25 ab	843.75 ab	1540.25 a	1554.50 ab
DMS*	233.81	822.92	1978.01	1530.49

[y]dat: Days after transplant. [Z] Means with the same letters are not statistically different (Tukey, ≤0.05). *Minimal Significant Difference ($P \leq 0.05$).

Number of females per gram of stained root. At 90 days after transplanting, the treatment with *T. asperellum* (Ta13-17) reduced the number of females per gram of stained root by 75% compared with the control. A similar effect was observed with the combination *T. asperellum* (Ta13-17) / *T. erinaceum* (Te10-15), which maintained nematode reproduction control through 122 days after transplanting. By the end of the crop cycle (137 days after transplanting), the combination (Ta13-17 and Te10-15) reduced the number of females per gram of stained root by 77.31% and 73.65% compared with the control and Vydate®, respectively (Table 3). In another study with tomato, combinations of *T. simmonsii* (Th09-06) / *T. harzianum* (Th33-59), *T. virens* (Th27-08) / *T. harzianum* (Th33-59), and *T. virens* (Th43-13) / *T. ghanense* (Th26-52) reduced reproduction by 90.1%, 88.1%, and 31.5%, respectively (Moo-Koh *et al.*, 2018). At the end of the crop cycle, the individual treatment with *T. asperellum* (Ta13-17) reduced reproduction by 43.81% compared with the control.

Table 3. Effect of treatments on the number of *M. incognita* females in organic cultivation of *C. chinense*.

Treatmet	Number of females per gram of dyed root			
	56 dat[y]	90 dat	122 dat	137 dat
T. asperellum (Ta13-17)	4.50 ab [z]	4.50 a	25.75 a	27.25 ab
T. erinaceum (Te10-15)	11.25 a	13.25 a	29 a	35.25 ab
Combination	0 b	4.50 a	6. 25 a	11 b
Control	8.50 ab	18 a	41.77 a	48. 5 a
Vydate®	7. 50 ab	37 a	32. 50 a	41.75 ab
DMS*	10.98	33.79	35.65	34. 43

[y]dat: Days after transplant. [Z] Means with the same letters are not statistically different (Tukey, ≤0.05). [*]Minimal Significant Difference ($P \leq 0.05$).

It has also been suggested that the properties of *Trichoderma* lie in its ability to parasitize different life stages of *M. incognita* (Sharon *et al.*, 2011). In particular, *T. asperellum* improves the tolerance of nematode-infected plants by enhancing biochemical and physiological traits, especially the production of secondary metabolites such as phenolic compounds, which hinder nematode reproduction (Saharan *et al.*, 2023).

Effect of microbial control on crop performance. For the agronomic variables, although no statistically significant differences were observed among treatments, greater effects on plant height, foliar biomass, and root volume were recorded with the combination of *Trichoderma* strains (Ta13-17 and Te10-15), with averages of 30.85 cm, 95.49 g, and 55.06 cm^3, respectively, compared with the individual application of *Trichoderma* spp. strains. The lowest plant height was observed with the Vydate® treatment, averaging 23.64 cm. Notably, the combination of *Trichoderma* strains (Ta13-17 and Te10-15) also had a positive effect on stem diameter (Table 4).

Application of *Trichoderma* spp. strains, studied as antagonists of *M. incognita*, suppressed nematode populations and reduced galling, which tended to improve crop growth, as reflected in the agronomic variables of habanero pepper. Growth promotion occurs through a specific interaction with *Trichoderma* and the production of indole-3-acetic acid, resulting in increased plant biomass (Contreras *et al.*, 2009; Martínez *et al.*, 2011). However, this response does not occur when the host interacts with a pathogen. In such cases, biocontrol microorganisms like *Trichoderma* redirect resources to activate defense mechanisms known as induced systemic resistance, through jasmonic acid, salicylic acid, or ethylene pathways, which explains the outcomes observed in this study (Hermosa *et al.*, 2013; Nawrocka *et al.*, 2013).

Table 4. Effect of treatments in the control of *M. incognita* on the agronomic variables of the organic cultivation of *C. chinense*.

Treatment	Plant height (cm)	Stem diameter (mm)	Foliar Biomass (g)	Root Volume (cm^3)
T. asperellum (Ta13-17)	28.84±2.68 a	7.84±0.82 ab	81.9±13.54 a	48.94±6.3 a
T. erinaceum (Te10-15)	26.76±1.36 a	7.16±0.75 ab	88.43±15.96 a	49.31±8.35 a
Combination	30.85±3.14 a	7.96±0.71 a	95.49±11.83 a	55.06±7.05 a
Control	30.41±1.26 a	7.47±0.67 ab	76.41±11.46 a	40.88±6.24 a
Vydate®	23.64±2.94 a	6.72±0.82 b	79.88±12.83 a	44.38±7.76 a
DMS*	9.32	1.23	52.26	16.00

[Z] Means with the same letters are not statistically different (Tukey, ≤0.05). [*]Minimal Significant Difference ($P \leq 0.05$).

Effect of treatments on fruit yield through the control of *M. incognita* in the organic cultivation of *C. chinense*. Yield and fruit size were evaluated across eight harvests, and analysis of variance showed significant differences among treatments ($p \leq 0.01$). The highest yields were obtained with treatments inoculated with *Trichoderma* spp. The treatments with *T. asperellum* (Ta13-17) and the combination of strains (Ta13-17 and Te10-15) not only outperformed the untreated control but also exceeded the nematicide treatment with Vydate®. In the first case, yield reached 244.78 g $plant^{-1}$, and in the second, 231.08 g $plant^{-1}$. The largest fruit size was recorded in the treatment with *T. erinaceum* (Te10-15) (Table 5). Some studies suggest that microbial antagonists may also reduce crop yield due to fungal competition (Meyer *et al.*, 2001), interactions with crop-associated fungi (Harman, 2006), or root suppression, factors that should be considered in future biocontrol studies. However, such an effect was evident in this study (Table 5).

Table 5. Effect of treatments on fruit yield in *C. chinense* cultivation.

Treatment	Yield (g $plant^{-1}$)	Number of fruits	Equatorial Diameter (cm)	Polar Diameter (cm)
T. asperellum (Ta13-17)	244.78±20.95 a	23.59±1.85 a	2.89±1.02 b	4.63±1.64 b
T. erinaceum (Te10-15)	210.4±17.65 ab	20.97±1.69 a	4.51±1.16 a	11.23±3.97 a
Combination	231.08±18.41 a	18.94±1.27 ab	2.89±1.02 b	3.94±1.39 b
Control	121.85±11.88 c	12.91±1.29 b	2.94±1.04 b	3.94±1.39 b
Vydate®	151.93±15.78 bc	17.59±1.81 ab	3.05±1.08 b	4.79±1.69 b
DMS*	66.47	6.18	0.45	1.13

[Z] Means with the same letters are not statistically different (Tukey, ≤0.05). [*]Minimal Significant Difference ($P \leq 0.05$).

CONCLUSIONS

Treatments that included inoculations with *T. asperellum* (Ta13-17) improved the control of *M. incognita*, reducing severity by 65.26%, while the combination of *Trichoderma* strains (Ta13-17 and Te10-15) achieved an 86.17% reduction. Nematode reproduction variables decreased by 75–85% in the number of eggs per gram of blended root and females per gram of stained root at 122 days after transplanting. Similarly, these treatments increased fruit production per plant by 40–50% compared with the control. The AUDPC and the apparent infection rate estimated with the Weibull model showed a significant reduction in the gall index with the individual application of *T. asperellum* (Ta13-17); however, when combined with *T. erinaceum* (Te10-15), gall formation was reduced even further.

Limitations

The experiments in this study were conducted under protected conditions, and the results must be validated in the field, since both biotic and abiotic factors influence the effectiveness of antagonists and the plant's response. It is recommended to detect genes involved in systemic resistance during the interaction between antagonistic fungi and the plant for nematode control.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Citlally Guadalupe Puc-Flores; execution of experiments, sampling, and measurement of variables. **Felicia Amalia Moo-Koh**; activation of fungal isolates, preparation of treatments, and manuscript drafting. **José María Tun Suárez**; critical review of the experiment and manuscript drafting process. **Eduardo Villanueva-Couoh**; design and guidance of the experiments and variable measurements, manuscript review, and final editing. **Jairo Cristóbal-Alejo**; study supervision, research analysis and design. Leader of the research project. Participated in data analysis, critical review of the manuscript, and its final approval.

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